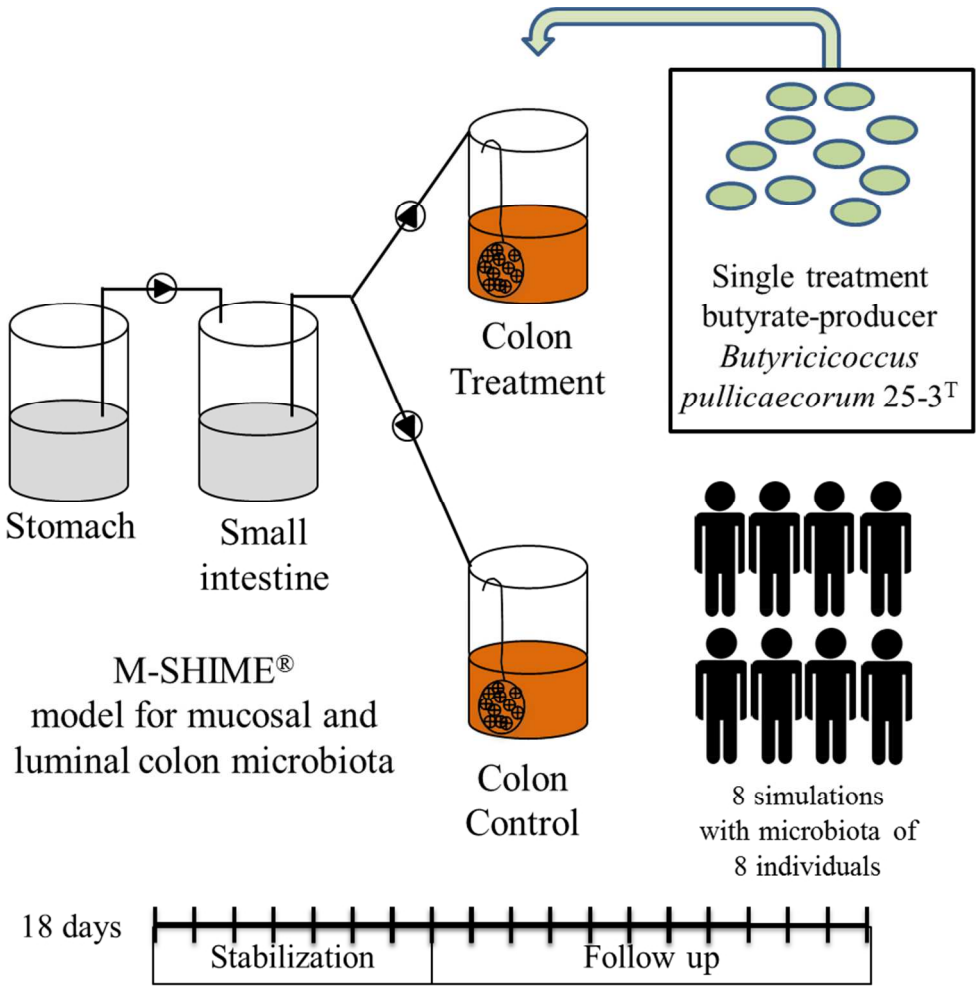
OXFORD UNIVERSITY PRESS | FEMS Microbiology Ecology

http://mc.manuscriptcentral.com/fems

Interindividual differences in response to treatment with butyrate-producing Butyricicoccus pullicaecorum 25-3T studied in an in vitro gut model

Journal:	*FEMS Microbiology Ecology*
Manuscript ID:	FEMSEC-15-03-0115
Manuscript Type:	Research article
Date Submitted by the Author:	12-Mar-2015
Complete List of Authors:	Geirnaert, Annelies; Ghent University, Laboratory of Microbial Ecology and Technology Wang, Jun; KU Leuven, Laboratory of Molecular Bacteriology (Rega Institute); VIB, Center for the Biology of Disease Tinck, Magali; Ghent University, Laboratory of Microbial Ecology and Technology Steyaert, Alix; Ghent University, Laboratory of Microbial Ecology and Technology Van den Abbeele, Pieter; Ghent University, Biochemical and Microbial Technology Eeckhaut, Venessa; Ghent University, PAthology, Bacteriology and Avian Diseases Vilchez-Vargas, Ramiro; Ghent University, Laboratory of Microbial Ecology and Technology Falony, Gwen; KU Leuven, Laboratory of Molecular Bacteriology (Rega Institute); VIB, Center for the Biology of Disease Laukens, Debby; Ghent University, Department of Gastroenterology De Vos, Martine; Ghent University, Gastroenterology van immerseel, filip; Ghent University, PAthology, Bacteriology and Avian Diseases; Raes, Jeroen; KU Leuven, Laboratory of Molecular Bacteriology (Rega Institute); VIB, Center for the Biology of Disease; Vrije Universiteit Brussel, Microbiology Unit Boon, Nico; Ghent University, Laboratory of Microbial Ecology and Technology Van de Wiele, Tom; University Gent, LabMET
Keywords:	Probiotic, Colonization, Gastrointestinal microbial ecology, in vitro, Butyric acid, Anaerobe



Graphical abstract: Butyrate-producing bacteria are promising probiotic candidates to maintain gastrointestinal health. Here, the behavior and butyrogenic effect of such a candidate was assessed in a model for the gut microbiota.
169x166mm (150 x 150 DPI)

Manuscript title

Interindividual differences in response to treatment with butyrate-producing *Butyricicoccus pullicaecorum* 25-3^{T} studied in an *in vitro* gut model

Running title

Butyrogenic effect of *B. pullicaecorum* in gut model M-SHIME

Annelies Geirnaert [a], Jun Wang [b, c], Magali Tinck [a], Alix Steyaert [a], Pieter Van den Abbeele[a], Venessa Eeckhaut [d], Ramiro Vilchez-Vargas [a], Gwen Falony [b, c], Debby Laukens [e], Martine De Vos [e], Filip Van Immerseel [d], Jeroen Raes [b, c, f], Nico Boon [a] and Tom Van de Wiele [a*]

[a] Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, 9000 Ghent, Belgium;

[b] Laboratory of Molecular Bacteriology (Rega Institute), KU Leuven, O&N I Herestraat 49, 3000 Leuven, Belgium

[c] VIB Center for the Biology of Disease, Herestraat 49, B-3000 Leuven, Belgium

[d] Department of Pathology, Bacteriology and Avian Diseases, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

[e] Department of Gastroenterology, Ghent University, 9000 Ghent, Belgium

[f] Microbiology Unit, Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium

* Corresponding author: Tom Van de Wiele, LabMET – UGent. Coupure Links 653, B-9000 Ghent, Belgium. Phone: +32 9 264 59 76. Fax: + 32 9 264 62 48. E-mail: tom.vandewiele@ugent.be

ABSTRACT

Butyrate-producing bacteria are promising probiotic candidates to target microbial dysbiosis in gastrointestinal disorders like Inflammatory Bowel Diseases. *Butyricicoccus pullicaecorum* 25-3^T, a butyrate-producing clostridial cluster IV strain, is such a candidate. Little is known about its abundance in the colon microbiota and its butyrogenic properties. We used the M-SHIME®, an *in vitro* simulator for the human intestinal microbial ecosystem, to study the effect of supplementing a single dose of *B. pullicaecorum* 25-3^T on lumen- and mucus-associated microbiota of eight individuals.

B. pullicaecorum was more abundant in mucus-associated microbiota compared with lumen microbiota. Supplementation with a single dose of *B. pullicaecorum* 25-3^T resulted in a temporary increase in *B. pullicaecorum* bacteria in luminal compartment of all donors. In two cases, an increased butyrate production was observed as compared with the control. 16S rRNA gene amplicon sequencing revealed the microbiota of responders to be different as compared to non-responder microbiota. We can conclude that *B. pullicaecorum* 25-3^T is a mucus-associated bacterium whose potency to stimulate butyrate production is characterized by a large interindividual variability in terms of composition of the receiving microbial community.

KEY WORDS

Colonization, probiotic, gastrointestinal microbial ecology, *in vitro*, butyric acid, anaerobe

1. INTRODUCTION

The human gastrointestinal tract contains up to 100 trillion (10^{14}) microbes which live in homeostatic symbiosis with their host and contribute to its health (Bäckhed, *et al.*, 2005). Microbial dysbiosis is defined as a shift of the microbial composition and activity from a normal, beneficial state to one that could influence human health and contribute to disease (Frank, *et al.*, 2011, Walker & Lawley, 2013). The onset and duration of several intestinal and systemic disorders have been linked to microbial dysbiosis (Alonso & Guarner, 2013, Walker & Lawley, 2013). This is also the case for Inflammatory Bowel Diseases (IBD), including Crohn's Disease (CD) and Ulcerative Colitis (UC), which are characterized by a chronic, relapsing inflammation of the gastrointestinal tract. Microbial dysbiosis in IBD can be described by a loss in diversity of the dominant bacterial phyla (particularly Firmicutes), increased numbers of Proteobacteria and Actinobacteria and reduced numbers of anaerobic Firmicutes bacteria (Manichanh, *et al.*, 2006, Frank, *et al.*, 2007, Willing, *et al.*, 2010, Lepage, *et al.*, 2011). The reduction in Firmicutes bacteria is due to the loss of butyrate-producing bacteria from clostridial cluster IV and XIVa. Butyrate is important to maintain gastrointestinal health, because it serves as the main energy source for gut epithelial cells, enhances epithelial barrier integrity and inhibits inflammation (Hamer, *et al.*, 2008). Therefore, it has been suggested to target microbial dysbiosis by supplementing butyrate-producing bacteria to restore homeostasis and health in IBD (Van Immerseel, *et al.*, 2010).

Butyricicoccus pullicaecorum 25-3^T is a butyrate-producing strain of the family Ruminococcaceae (clostridial cluster IV) with potential probiotic characteristics (Eeckhaut, *et al.*, 2008). While the genus *Butyricicoccus* is decreased in abundance in stool samples of IBD patients, *B. pullicaecorum* 25-3^T is able to attenuate chemically induced colitis in a rodent IBD model (Eeckhaut, *et al.*, 2012). In an i*n vitro* study, we demonstrated a good intrinsic

tolerance of *B. pullicaecorum* 25-3^T to stomach and small intestinal conditions which makes it suitable for probiotic application (Geirnaert, *et al.*, 2014).

To further assess the probiotic use of *B. pullicaecorum* 25-3^T, it is important to know its behavior in the presence of a complex microbial community under colon conditions. *In vitro* models which simulate the human microbiota are a good tool to study the change in composition and metabolic activity after treatment with a probiotic, prebiotic, or other compound without the influence of the host (Venema & van den Abbeele, 2013). The M-SHIME® is such a model which simulates the mucosal and luminal human intestinal microbial ecosystem (Van den Abbeele, *et al.*, 2012). The advantages of the M-SHIME® over other common *in vitro* models are the incorporation of a mucus environment, conservation of butyrate-producing bacteria, and maintenance of interindividual differences in composition and activity of the microbiota *in vitro* (Van den Abbeele, *et al.*, 2013). It has previously been used to study the colonization of microbiota of UC patients (Vermeiren, *et al.*, 2012, Vigsnaes, *et al.*, 2013) .

Here, we use the M-SHIME® to assess the colonization potential of *B. pullicaecorum* 25-3^T and to analyze its impact on butyrate production by the microbiota of different individuals (healthy volunteers and CD patients in remission) after *B. pullicaecorum* 25-3^T administration. We applied a single dose of *B. pullicaecorum* 25-3^T to monitor its growth or wash-out from the microbial communities during the 10 day follow-up period and effect of composition of receiving microbial community.

2. MATERIALS AND METHODS

2.1. Bacterial strain, growth conditions and preparation treatment

Butyricicoccus pullicaecorum 25-3^T (LMG 24109^T) was grown in anaerobic M2GSC medium at pH 6 prepared as described by Miyazaki et al. (Miyazaki, *et al.*, 1997) but with

15% (v/v) of clarified rumen fluid instead of 30% (v/v). M2GSC agar (1.5% w/v) plates were incubated at 37°C in an anaerobic (10% CO_2, 90% N_2) workstation (GP-Campus, Jacomex, TCPS NV, Rotselaar, Belgium) for 20h. Before use in each experiment, a *B. pullicaecorum* colony was transferred into 10 mL of anaerobic M2GSC broth and incubated overnight at 37°C. Subsequently, the culture was subcultured (10% v/v) once in 500 mL anaerobic M2GSC broth and incubated for 20h at 37°C. The *B. pullicaecorum* culture was concentrated from 500 mL to 10 mL by centrifugation (10 min, 1500 g). The supernatant was removed and the pellet was resuspended in 10 mL anaerobic phosphate buffered saline (PBSS per L: 8.8g K_2HPO_4, 6.8g KH_2PO_4, 8 g NaCl, 1 g cysteine-HCl).

2.2. Fecal bacteria from human volunteers

Fecal bacteria of 3 healthy individuals (HV 1 – HV 3, aged 23-37) and 5 CD patients in remission (no active inflammation) for more than 12 months (CD 1 – CD 5, aged 24-41) were prepared to inoculate M-SHIME. The study was approved by the Ethics Committee of the University Hospital Ghent (permit numbers EC UZG 2006/377 & EC UZG 2012/415), and all volunteers received and signed an informed consent form. None of the donors had received antibiotics or probiotics for at least 3 months before fecal sample donation. CD 1 – CD 3 received maintenance treatment with azathioprine (immunosuppressive drug) and CD 4 and CD 5 had not taken any medication since 8 months before sample donation. CD 2, CD 4, and CD 5 had a history of ileitis, CD 1 and CD 3 had a history of ileocolitis.

Fecal samples were collected in airtight containers together with one AnaeroGen sachet (Oxoid) to maintain anoxic conditions until start of incubation. Time between fecal sample donation and start of incubation was maximum 2h. A 20% (m/v) fecal suspension was prepared by homogenizing the fecal sample with 0.1 M anaerobic phosphate buffer (per L: 8.8g K_2HPO_4, 6.8g KH_2PO_4 and 1g $C_2H_3O_2Sna$, pH 6.8) in a stomacher for 2 min. After

removing particulate material by centrifugation (2 min. at 500g) the suspension was used as inoculum for incubation.

2.3. Simulation of luminal and mucosal microbiota – M-SHIME®

The behavior of *B. pullicaecorum* was studied in the M-SHIME®, a dynamic *in vitro* model which simulates the mucosal and luminal human intestinal microbial ecosystem (ProDigest-Ghent University, Ghent, Belgium) (Van den Abbeele, *et al.*, 2012, Van den Abbeele, *et al.*, 2013). This model consists of pH controlled, stirred (200 rpm), airtight, double-jacketed glass vessels kept on 37°C and under anaerobic conditions by daily flushing with N_2 (15 min). The setup used in this study consisted of a stomach and a small intestine vessel and two colon vessels (control and treatment) in parallel for each studied donor (Figure 1). The system was operated and simulation media were prepared as described earlier (Van den Abbeele, *et al.*, 2013). The colon vessels were inoculated at the start with 40 mL fecal suspension in 500 mL nutritional medium. After an initial static incubation of 18 h, three times a day 140 mL nutritional medium and 60 mL pancreatic juice per colon vessel were supplemented to the small intestine vessel. Small intestine simulation suspension was divided over the colon vessels. The residence time in the colon vessels was 20 h and pH was controlled at pH 6.15- 6.40. To simulate mucosal microbiota, 60 mucin agar-covered microcosms in a polyethylene netting were added to each colon vessel. Mucin agar consisted of 5% (m/v) commercial pig gastric mucin and 1% (m/v) agar. Every two to three days 2/3 of the mucin agar-covered microcosms were replaced by fresh sterile ones under a flow of N_2 to prevent disruption of anaerobic conditions. Seven days after inoculation, colon vessels were inoculated with 10 mL *B. pullicaecorum* culture (10^9 bacteria/mL) (treatment vessels) or 10 mL sterile anaerobic PBSS (control vessels). After treatment there was a follow-up period of 10 days. Luminal samples were taken every day, mucin agar samples were taken every two to three days. Mucin agar-covered microcosms were washed with sterile PBSS to remove lumen

bacteria. Mucin agar was removed from microcosms, homogenized and stored immediately at -20°C until further analysis.

Previous studies showed a good reproducibility of the SHIME (Van den Abbeele, *et al.*, 2010). To validate the reproducibility during this study with an M-SHIME, we used the fecal slurry of 2 individuals (IBD 5 and HV 3) to inoculate each two identical M-SHIME colon vessels. SCFA concentrations in lumen samples of replicates were similar during the experiment of 17 days and confirmed reproducibility (Supplementary Figure 1).

2.4. Short-Chain Fatty Acid (SCFA) analysis

The SCFA in the luminal samples of the M-SHIME® were extracted with diethyl ether and analyzed using a gas chromatograph as described by De Weirdt et al. (De Weirdt, *et al.*, 2010). The concentration in mM of acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, caproate and isocaproate was determined in each sample. The concentration of acetate and butyrate was expressed as mol% which is the ratio of the concentration of acetate or butyrate (mM) and the total SCFA concentration (mM) multiplied by 100 in the sample.

2.5. DNA extraction

Liquid samples (1 mL fecal suspension and 1 mL lumen M-SHIME®) for total DNA extraction were centrifuged for 10 min at maximum speed, supernatant was removed and pellet was stored immediately at -20°C until further analysis.

Total DNA was extracted from pellet of 1 mL liquid samples and 0.5 g mucin agar following a protocol adapted from Vilchez-Vargas et al. (Vilchez-Vargas, *et al.*, 2013). Cells were lysed with 1 mL lysis buffer (100 mM Tris/HCl pH 8.0, 100 mM EDTA pH 8, 100 mM NaCl, 1% (m/v) polyvinylpyrrolidone and 2% (m/v) sodium docecyl sulphate) and 200 mg glass beads (0.11 mm, Sartorius) in a FastPrep®-96 instrument (MP Biomedicals, Santa Ana, USA) for two times 40 s (1600 rpm). After removing glass beads by centrifugation (5 min at maximum speed), DNA was extracted from supernatant following a phenol-chloroform

extraction. DNA was precipitated with 1 volume ice-cold isopropyl alcohol and 0.1 volume 3 M sodium acetate for at least 1 h at -20°C. After removal of isopropyl alcohol by centrifugation (30 min, maximum speed) the DNA pellet was dried and resuspended in 100 µL (fecal sample) or 30 µL (M-SHIME® sample) 1x TE (10 mM Tris, 1 mM EDTA) buffer. After finishing the extraction protocol, DNA samples were immediately stored at -20°C until further analysis.

Quality of DNA samples was analyzed by 1% (w/v) agarose (Life technologies™, Madrid, Spain) gel electrophoresis. DNA was quantified by a fluorescence assay with the QuantiFluor® dsDNA kit (Promega, Madison, USA) and Glomax®-Multi+ system (Promega, Madison, USA).

2.6. qPCR

Total bacterial 16S rRNA gene and species specific 16S rRNA gene of *B. pullicaecorum* was quantified with qPCR in 100-fold diluted DNA extracts of fecal and M-SHIME® samples. All qPCR assays were performed on a StepOnePlus™ Real-Time PCR system (Applied Biosystems, Carlsbad, CA). The amplification reactions were carried out in triplicate in a volume of 25 µL which contained 20 µL of in-house prepared mastermix and 5 µL of DNA template. The in-house prepared mastermix was comprised of 1x Colorless GoTaq® reaction buffer (Promega), 1.5 mM $MgCl_2$, 200 nM dNTP mix, 200 nM of each forward and reverse primer, 0.625 U GoTaq® HotStart polymerase (Promega) and 0.1x SYBR® Green I (Invitrogen™, provided at 10 000x, stock solutions of 20x were prepared in DMSO). Primers for total Bacteria (PRBA 338f and 518r) amplified a 180 bp amplicon of V3 region of the 16S rRNA gene (Ovreas*, et al.*, 1997). Cycling program for total bacteria was as follows: 3 min at 95°C followed by 40 cycles of 1 min at 95°C, 40 s at 56°C and 40 s at 72°C. Species specific primers were designed and synthesized by PrimerDesign Ltd. (Southampton, UK) and amplified a 126 bp amplicon of the 16S rRNA gene of *B. pullicaecorum.* Primer

sequences of forward primer was 5'- GAGGCAGCAGTGGGGAA and reverse primer 5'-TCTTCAGGTACCGTCATTTGTT. Program was as follows: 3 min at 95°C followed by 40 cycles of 1 min at 95°C, 40 s at 54.5°C and 40 s at 72°C.

2.7. Illumina

The V3-V4 region of the 16S rRNA gene was amplified with primer pair 341F and 785R, with dual multiplex identifier (MID) and adaptors as described by Kozich et al (2013, AEM). Sequencing was performed using Illumina MiSeq sequencer and sequencing kit MiSeq V3 to produce 300 bp pair-end reads. After de-multiplexing, fastq sequences were merged using FLASH (Magoc & Salzberg, 2011) software with default parameters, and successfully combined reads were filtered based on quality (>90% of nucleotides must have quality score 30 or higher for every read) using Fastx tool kit (http://hannonlab.cshl.edu/fastx_toolkit/). A minimum of 3,000 reads was obtained for each sample included in the study. Chimeras were removed using UCHIME (Egdar et al 2013) and each sample was standardized to 3,000 reads using random selection of reads. The taxonomy of reads was determined using RDP classifier (Wang et al 2007) and taxonomy tables were created using Perl scripts.

2.8. Statistical analysis

All statistical analyses were performed in R. Bray-Curtis dissimilarity was the major beta-diversity measure used in this study and was calculated using package "vegan" (Dixon 2003); alpha diversity measures included Chao1 richness measurement, observed number of genera/OTUs and Shannon evenness, all calculated in "vegan" as well. "adonis" (analysis of dissimilarity, multidimensional ANOVA of distance matrices) was used to determine the variation explained by different factors tested in the study, as well as the significances. Constrained analysis of principal coordinates ('capscale', Anderson & Willis, 2003) was used

to perform coordination of samples according to the variable being tested and determine the relative importance of difference variables.

3. RESULTS

3.1. Relative B. pullicaecorum abundance increased in the mucosal microbial community compared with luminal microbial community of the M-SHIME®

Colon vessels of the M-SHIME® were inoculated with fecal suspensions of one individual and in total eight incubations were performed with fecal suspensions of eight different individuals (CD1-5; HV1-3). During a period of seven days, fecal bacteria were able to colonize the lumen and/or mucus environment of the *in vitro* model. The concentration of bacteria (total 16S rRNA gene) and indigenous *B. pullicaecorum* (species-specific 16S rRNA gene assay) was determined by qPCR to determine the initial levels of *B. pullicaecorum* before supplementation. Luminal concentrations of total bacteria on day 7 ranged from 7.6 to 9.0 log copies/mL with a median at 8.5 log copies/mL while mucosal concentrations ranged from 8.9 to 9.9 log copies/g with a median of 9.4 log copies/g. Luminal concentrations of *B. pullicaecorum* varied from 4.3 to 6.2 log copies/mL with a median at 5.5 log copies/mL while mucosal concentrations ranged from 6.3 to 7.8 log copies/mL with a median at 7.2 log copies/mL. Overall, there was no difference in concentration of total and *B. pullicaecorum* bacteria between CD and HV microbiota in the M-SHIME (data not shown). The ratio of *B. pullicaecorum* 16S rRNA gene copies to total 16S rRNA gene copies was used to calculate the relative abundance of *B. pullicaecorum* in the luminal versus mucosal microbiome. *B. pullicaecorum* was relatively more abundant ($p<0.0001$) in mucosal microbial community (0.85 ± 0.14 %) than the luminal microbial community (0.16 ± 0.03 %) (Figure 2A).

3.2. Treatment of microbial communities with single dose B. pullicaecorum 25-3^T

On day seven, the treatment vessels were supplemented with a single dose of *B.*

pullicaecorum 25-3^{T} (on average 9 log copies/mL M-SHIME® suspension). Before supplementation, there was no difference in average concentrations of total bacteria and *B. pullicaecorum* in lumen and mucus samples of treated vessels compared with control vessels (Supplementary Figure 2 and Figure 2. B1). Three days after treatment the mean concentration of *B. pullicaecorum* was higher ($p = 0.005$) in lumen of treated vessels compared with control vessels (Figure 2.B2). This was not the case in mucus samples. If we compare treatment with control concentrations in each individual microbial community there were more *B. pullicaecorum* in mucus samples of treated vessels compared with control in 4 out of 8 cases (Supplementary Figure 3). Ten days after supplementation *B. pullicaecorum* concentration in the treated vessels was similar to those in the control vessels (Figure 2.B3). Based on 16S rRNA gene amplicon sequencing, we observed a clear increase in relative abundances of *Butyricicoccus* spp. in treated lumen and mucus samples of CD 1, CD 4, CD 5, HV 1, HV 2 and HV 3 three days after treatment (Supplementary Table S1). Seven days after treatment, *Butyricicoccus* was still higher in mucus samples of treated vessels compared to control of CD 4, CD 5, HV 1, HV 2 and HV 3.

3.3. SCFA profiles show inter-individual differences after treatment with B. pullicaecorum 25-3^{T}

Short-Chain Fatty Acid (SCFA) concentrations were determined in lumen samples to evaluate the metabolic activity of the different microbial communities in the M-SHIME®. The mean concentrations of total SCFA during the startup period (day 3- day 7) ranged from 38.6 (± 2.8) to 58.5 (± 4.2) mM in the 16 M-SHIME® vessels (Table 1). There were inter-individual differences in the SCFA profile. The relative concentration of acetate/propionate/butyrate for example ranged from 50%/20%/20% (CD 3, control) to 73%/13%/11% (HV1, control). Higher inter-individual differences were observed in case of branched chain fatty acids (BCFA) levels (1% to 10%). There was no difference in SCFA

profiles between control and treatment vessels of each corresponding donor microbial community. SCFA profiles of CD microbiota were comparable to those of HV microbiota in the M-SHIME. On average, single dose of *B. pullicaecorum* 25-3^T did not increase SCFA production during a ten days follow-up period (Table 1). In two cases (CD 5 and HV 1), a clear difference in SCFA profile was observed between treatment and control (Figure 3). In case of donor CD 5, the relative concentration of acetate started to decrease after 4 days of supplementation to 53% (day 14, control level 74%), whereas, the relative concentration of butyrate increased to a level of 18% (day 14, control level 8%). In case of donor HV 1, the relative concentration of acetate decreased to 53% (day 13, control level 71%) and the relative concentration of butyrate increased to 22% (day 13, control level 7%).

3.4. Responder microbiota different from non-responder microbiota

To investigate the basis of differences in butyrate/acetate production, we defined groups of "responders" (RS, samples that responded to the inoculation and showed increase in butyrate production compared to controls without inoculations, i.e. CD5 and HV1) and "non-responders" (NR, the rest). We did not find significant differences in alpha-diversity between NR and RS samples (Wilcox test $p>0.05$ in lumen and mucus). However, significant differences were observed between RS and NR samples from the control lumen (21.9% variation in genus, $p=0.002$) and mucus (23.6% variation of genus, $p=0.002$, Figure 4) were found, indicating a distinct profile for non-treated RS microbiome. We analysed the major genera (those with average abundance >0.5% in the control samples, n=24) using Wilcox test and discovered four genera with significant differences ($p<0.05$) between the NR and RS control samples in the lumen and seven in the mucus (Figure 5).

In the NR group no significant differences were found between samples after treatment and control (all $p>0.05$), nor in the mucus samples of the RS samples; nevertheless, significant differences were found between the lumen samples of the treatment and control in

the RS group (33.5% variation in genus variation, p=0.035). We examined the major genera (defined using similar criteria as above, n=21) using Wilcox test between the RS control samples and treatment samples, where we found three genera exhibiting significant differences between control and treatment (Figure 6). A significant increase of *Anaeroglobus* and *Sutterella* was found in the RS after treatment, while *Citrobacter* decreased, and no significant changes were found in the NR (Figure 6).

4. DISCUSSION

The objective of this study was to assess the *in vitro* colonization preference of indigenous *B. pullicaecorum* from eight individuals and evaluate the colonization ability of a *B. pullicaecorum* isolate 25-3^T in the luminal and mucosal microbiome after a single supplementation. We further assessed whether a single dose of *B. pullicaecorum* 25-3^T resulted in increased butyrate production by the *in vitro* simulated gut microbiome. Finally, the effect of the inoculation of *B. pullicaecorum* 25-3^T on the microbiota composition was analyzed.

B. pullicaecorum was more abundant in mucin- associated microbiota of the M-SHIME which is a first indication that it will also associate with the mucus layer in the gut. This confirms previous studies where the genus *Butyricicoccus* was detected in human mucosal biopsy samples, Nava et al. reported *Butyricicoccus* at mean relative abundances of 1-2% (Frank, *et al.*, 2011, Nava & Stappenbeck, 2011, Durbán, *et al.*, 2012, Harrell, *et al.*, 2012, Ng, *et al.*, 2013). This corresponds with the mean relative abundance of *B. pullicaecorum* in the mucin-associated microbiota of 0.85% in our study. Association with the intestinal mucosa is a key characteristic in selection of novel probiotic bacteria because it is important for immune modulation, resistance to pathogen colonization, enhanced mucosal healing and prolonged residence time in the gut (Ouwehand, *et al.*, 2002). The relative abundance of *B.*

pullicaecorum in lumen M-SHIME samples (0.02 % - 0.34 %) was comparable with previously reported relative abundances of *Butyricicoccus* spp. in human fecal samples (0.05% - 0.4%) (Claesson, *et al.*, 2012, Schnorr, *et al.*, 2014). We conclude that *Butyricicoccus* has affinity for colonizing the mucosal environment.

Supplementation with a single dose of *B. pullicaecorum* 25-3^T resulted in a temporary increase in *B. pullicaecorum* bacteria in lumen microbiota of all individuals. The difference in *B. pullicaecorum* concentration between treated and non-treated colon compartments was gone ten days after treatment. This indicates that *B. pullicaecorum* 25-3^T is only able to temporarily colonize the microbiota after a single supplementation. The persistence of traditional probiotics, *Lactobacillus* and *Bifidobacterium* species, is also generally low (Lawley & Walker, 2013). For example, in a trial with *Lactobacillus reuteri*, the supplemented strain was no longer detected in the majority of participants four days after stopping the treatment (Rattanaprasert, *et al.*, 2014). To successfully colonize the gut microbiota, the supplemented species has to compete with the established resident microbiota for niches and nutrients (Lawley & Walker, 2013). The mucosal niche is thought to be saturable and will already contain a specific population (Gibson, *et al.*, 2014), which makes it less obvious for exogenous strains to colonize that niche. The colonization resistance of the resident microbiota towards exogenous strains can be obtained through physical exclusion and/or production of antimicrobials, but will also depend on interindividual differences in composition of the endogenous microbiome.. For example, in a double blinded, placebo-controlled crossover study with vaginal administration of two probiotic *Lactobacillus* strains, the supplemented strains were only detected in 7 of 12 cases after probiotic treatment (Bisanz, *et al.*, 2014). Such studies correspond with our finding of a variable success in *B. pullicaecorum* 25-3^T colonization in the eight human-derived microbiota.

We identified two responder microbiota that displayed a clear increase in butyrate levels upon the single supplementation of *B. pullicaecorum* 25-3^T, compared to non-treated microbiota. It is noteworthy that responder microbiota already displayed lower butyrate levels than non-responder microbiota prior to the treatment. Interestingly, the increase in butyrate levels for the responder microbiota was concomitant with a decrease in acetate levels. This indicates the presence of cross-feeding which is the metabolic interaction between acetate-consuming and butyrate-producing microbiota (De Vuyst & Leroy, 2011). It is known that many butyrate-producing bacteria, including *B. pullicaecorum*, follow the butyryl-CoA: acetate CoA-transferase pathway (Vital*, et al.*, 2014), which converts intra- and extra-cellular acetate and intracellular butyryl-CoA into butyrate and acetyl-CoA (Louis & Flint, 2009).

Only two out of eight microbiota were responsive to *B. pullicaecorum* supplementation in terms of butyrate production. Studies with murine models reported a general increase in butyrate production after treatment with butyrate-producing *Clostridium tyrobutyricum*, *Butyrivibrio fibrisolvens* or *Eubacterium limosum* (Okamoto*, et al.*, 2000, Asanuma*, et al.*, 2001, Ohkawara*, et al.*, 2005, Kanauchi*, et al.*, 2006, Possemiers*, et al.*, 2008, Hudcovic*, et al.*, 2012). However, the variability in response was not characterized as biological specimens from different animals were pooled. It is not clear in our study whether higher butyrate levels in the responder microbiota result from a direct effect – butyrate production by supplemented *B. pullicaecorum* 25-3^T – or an indirect effect – stimulation of the butyrate-producing community by supplemented *B. pullicaecorum* 25-3^T. While the SHIME model has been successful in demonstrating increased butyrate production upon supplementation of the butyrate-producing *E. limosum* (Possemiers*, et al.*, 2008), the impact on the endogenous microbiome needs further characterization.

The original (non-treated) composition of luminal and mucosal microbiota from responders and non-responders revealed significant differences. A couple of genera were

significantly increased or decreased but no clear specific phylogenetic background was identified. With respect to the change in RS microbiota upon *B. pullicaecorum* treatment, *Anaeroglobus* and *Sutterella* were the two genera that significantly increased compared to the control. *Anaeroglobus* is a relatively new taxon and is reported to have the ability to produce butyrate; the butyrate producing ability of the *Sutterella* genus is less clear (Carlier*, et al.*, 2002). The genus *Sutterella* was recently described as one of the genera detected less frequently among pouchitis and Crohn's disease-like patients compared to non-inflamed control (Tyler*, et al.*, 2013). Phylogenetically different species in the human gut can perform similar (metabolic) functions and this leads to a functional core microbiome instead of a phylogenetic core microbiome (Lozupone*, et al.*, 2012). This functional redundancy makes it difficult to link the observed differences in composition of the responder microbiota to its function. Therefore, it is not yet clear what explains the effect of the treatment with *B. pullicaecorum* 25-3^T on butyrate production in the two responder microbiota.

We can conclude that *B. pullicaecorum* is an efficient colonizer of the mucus environment. While a single treatment with *B. pullicaecorum* 25-3^T did not result in a persistent colonization, it was effective in increasing mucosal *B. pullicaecorum* levels in four out of eight cases up to 10 days after treatment, and in stimulating butyrate production in two out of eight cases. While the original (non-treated) responder microbiota significantly differed from non-responder microbiota and shifts in the responder microbiome were noted upon *B. pullicaecorum* 25-3T administration, the factors for explaining the variability in response need to be investigated in studies with more individuals.

FUNDING

This work was supported by a research grant of "Strategisch Basisonderzoek – SBO" of the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT-Vlaanderen, project nr. 100016); a 'Geconcerteerde OnderzoeksActie' GOA-grant

(BOF12/GOA/008) from Ghent University and an award from the Belgian foundation for Crohn and ulcerative colitis patients (CCV-vzw) (research award 2012).

ACKNOWLEDGEMENTS

We thank Jana De Bodt , Tim Lacoere and Elien Glorieus for technical assistance.

REFERENCES

Alonso VR & Guarner F (2013) Linking the gut microbiota to human health. *British Journal of Nutrition* **109**: S21-S26.

Asanuma N, Kawato M & Hino T (2001) Presence of Butyrivibrio fibrisolvens in the digestive tract of dogs and cats, and its contribution to butyrate production. *J Gen Appl Microbiol* **47**: 313-319.

Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA & Gordon JI (2005) Host-Bacterial Mutualism in the Human Intestine. *Science* **307**: 1915-1920.

Bisanz JE, Seney S, McMillan A*, et al.* (2014) A Systems Biology Approach Investigating the Effect of Probiotics on the Vaginal Microbiome and Host Responses in a Double Blind, Placebo-Controlled Clinical Trial of Post-Menopausal Women. *Plos One* **9**: e104511.

Carlier JP, Marchandin H, Jumas-Bilak E, Lorin V, Henry C, Carriere C & Jean-Pierre H (2002) Anaeroglobus geminatus gen. nov., sp nov., a novel member of the family Veillonellaceae. *International Journal of Systematic and Evolutionary Microbiology* **52**: 983-986.

Claesson MJ, Jeffery IB, Conde S*, et al.* (2012) Gut microbiota composition correlates with diet and health in the elderly. *Nature* **488**: 178-184.

De Vuyst L & Leroy F (2011) Cross-feeding between bifidobacteria and butyrate-producing colon bacteria explains bifdobacterial competitiveness, butyrate production, and gas production. *International Journal of Food Microbiology* **149**: 73-80.

De Weirdt R, Possemiers S, Vermeulen G, Moerdijk-Poortvliet TCW, Boschker HTS, Verstraete W & Van de Wiele T (2010) Human faecal microbiota display variable patterns of glycerol metabolism. *Fems Microbiology Ecology* **74**: 601-611.

Durbán A, Abellán JJ, Jiménez-Hernández N*, et al.* (2012) Structural alterations of faecal and mucosa-associated bacterial communities in irritable bowel syndrome. *Environmental Microbiology Reports* **4**: 242-247.

Eeckhaut V, Van Immerseel F, Teirlynck E*, et al.* (2008) Butyricicoccus pullicaecorum gen. nov., sp nov., an anaerobic, butyrate-producing bacterium isolated from the caecal content of a broiler chicken. *International Journal of Systematic and Evolutionary Microbiology* **58**: 2799-2802.

Eeckhaut V, Machiels K, Perrier C*, et al.* (2012) Butyricicoccus pullicaecorum in inflammatory bowel disease. *Gut*.

Frank DN, Zhu W, Sartor RB & Li E (2011) Investigating the biological and clinical significance of human dysbioses. *Trends in Microbiology* **19**: 427-434.

Frank DN, Amand ALS, Feldman RA, Boedeker EC, Harpaz N & Pace NR (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 13780-13785.

Frank DN, Robertson CE, Hamm CM*, et al.* (2011) Disease Phenotype and Genotype Are Associated with Shifts in Intestinal-associated Microbiota in Inflammatory Bowel Diseases. *Inflammatory Bowel Diseases* **17**: 179-184.

Geirnaert A, Steyaert A, Eeckhaut V*, et al.* (2014) Butyricicoccus pullicaecorum, a butyrate producer with probiotic potential, is intrinsically tolerant to stomach and small intestine conditions. *Anaerobe* **30**: 70-74.

Gibson MK, Pesesky MW & Dantas G (2014) The Yin and Yang of Bacterial Resilience in the Human Gut Microbiota. *Journal of Molecular Biology* **426**: 3866-3876.

Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ & Brummer RJ (2008) Review article: the role of butyrate on colonic function. *Alimentary Pharmacology & Therapeutics* **27**: 104-119.

Harrell L, Wang Y, Antonopoulos D*, et al.* (2012) Standard colonic lavage alters the natural state of mucosal-associated microbiota in the human colon. *Plos One* **7**: e32545.

Hudcovic T, Kolinska J, Klepetar J*, et al.* (2012) Protective effect of Clostridium tyrobutyricum in acute dextran sodium sulphate-induced colitis: differential regulation of tumour necrosis factor-alpha and interleukin-18 in BALB/c and severe combined immunodeficiency mice. *Clinical and Experimental Immunology* **167**: 356-365.

Kanauchi O, Fukuda M, Matsumoto Y*, et al.* (2006) Eubacterium limosum ameliorates experimental colitis and metabolite of microbe attenuates colonic inflammatory action with increase of mucosal integrity. *World J Gastroenterol* **12**: 1071-1077.

Lawley TD & Walker AW (2013) Intestinal colonization resistance. *Immunology* **138**: 1-11.

Lepage P, Hasler R, Spehlmann ME*, et al.* (2011) Twin study indicates loss of interaction between microbiota and mucosa of patients with ulcerative colitis. *Gastroenterology* **141**: 227-236.

Louis P & Flint HJ (2009) Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *Fems Microbiology Letters* **294**: 1-8.

Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK & Knight R (2012) Diversity, stability and resilience of the human gut microbiota. *Nature* **489**: 220-230.

Manichanh C, Rigottier-Gois L, Bonnaud E*, et al.* (2006) Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut* **55**: 205-211.

Miyazaki K, Martin JC, Marinsek-Logar R & Flint HJ (1997) Degradation and Utilization of Xylans by the Rumen AnaerobePrevotella bryantii(formerlyP. ruminicolasubsp.brevis) B14. *Anaerobe* **3**: 373-381.

Nava GM & Stappenbeck TS (2011) Diversity of the autochthonous colonic microbiota. *Gut Microbes* **2**: 99-104.

Ng SC, Lam EFC, Lam TTY*, et al.* (2013) Effect of probiotic bacteria on the intestinal microbiota in irritable bowel syndrome. *Journal of Gastroenterology and Hepatology* **28**: 1624-1631.

Ohkawara S, Furuya H, Nagashima K, Asanuma N & Hino T (2005) Oral administration of butyrivibrio fibrisolvens, a butyrate-producing bacterium, decreases the formation of aberrant crypt foci in the colon and rectum of mice. *The Journal of Nutrition* **135**: 2878-2883.

Okamoto T, Sasaki M, Tsujikawa T, Fujiyama Y, Bamba T & Kusunoki M (2000) Preventive efficacy of butyrate enemas and oral administration of Clostridium butyricum M588 in dextran sodium sulfate-induced colitis in rats. *Journal of gastroenterology* **35**: 341-346.

Ouwehand A, Salminen S & Isolauri E (2002) Probiotics: an overview of beneficial effects. *Antonie van Leeuwenhoek* **82**: 279-289.

Ovreas L, Forney L, Daae FL & Torsvik V (1997) Distribution of bacterioplankton in meromictic Lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Applied and Environmental Microbiology* **63**: 3367-3373.

Possemiers S, Rabot S, Espin JC*, et al.* (2008) Eubacterium limosum activates isoxanthohumol from hops (Humulus lupulus L.) into the potent phytoestrogen 8-prenylnaringenin in vitro and in rat intestine. *Journal of Nutrition* **138**: 1310-1316.

Rattanaprasert M, Roos S, Hutkins RW & Walter J (2014) Quantitative evaluation of synbiotic strategies to improve persistence and metabolic activity of Lactobacillus reuteri DSM 17938 in the human gastrointestinal tract. *Journal of Functional Foods* **10**: 85-94.

Schnorr SL, Candela M, Rampelli S*, et al.* (2014) Gut microbiome of the Hadza hunter-gatherers. *Nat Commun* **5**.

Tyler AD, Knox N, Kabakchiev B*, et al.* (2013) Characterization of the gut-associated microbiome in inflammatory pouch complications following ileal pouch-anal anastomosis. *Plos One* **8**.

Van den Abbeele P, Belzer C, Goossens M*, et al.* (2013) Butyrate-producing Clostridium cluster XIVa species specifically colonize mucins in an in vitro gut model. *ISME J* **7**: 949-961.

Van den Abbeele P, Roos S, Eeckhaut V*, et al.* (2012) Incorporating a mucosal environment in a dynamic gut model results in a more representative colonization by lactobacilli. *Microbial biotechnology* **5**: 106-115.

Van den Abbeele P, Grootaert C, Marzorati M, *et al.* (2010) Microbial community development in a dynamic gut model is reproducible, colon region specific, and selective for Bacteroidetes and Clostridium cluster IX. *Appl Environ Microbiol* **76**: 5237-5246.

Van Immerseel F, Ducatelle R, De Vos M, *et al.* (2010) Butyric acid-producing anaerobic bacteria as a novel probiotic treatment approach for inflammatory bowel disease. *Journal of Medical Microbiology* **59**: 141-143.

Venema K & van den Abbeele P (2013) Experimental models of the gut microbiome. *Best Practice & Research in Clinical Gastroenterology* **27**: 115-126.

Vermeiren J, Van den Abbeele P, Laukens D, Vigsnaes LK, De Vos M, Boon N & Van de Wiele T (2012) Decreased colonization of fecal Clostridium coccoides/Eubacterium rectale species from ulcerative colitis patients in an in vitro dynamic gut model with mucin environment. *Fems Microbiology Ecology* **79**: 685-696.

Vigsnaes LK, van den Abbeele P, Sulek K, *et al.* (2013) Microbiotas from UC patients display altered metabolism and reduced ability of LAB to colonize mucus. *Scientific Reports* **3**.

Vilchez-Vargas R, Geffers R, Suarez-Diez M, *et al.* (2013) Analysis of the microbial gene landscape and transcriptome for aromatic pollutants and alkane degradation using a novel internally calibrated microarray system. *Environ Microbiol* **15**: 1016-1039.

Vital M, Howe AC & Tiedje JM (2014) Revealing the Bacterial Butyrate Synthesis Pathways by Analyzing (Meta)genomic Data. *Mbio* **5**.

Walker AW & Lawley TD (2013) Therapeutic modulation of intestinal dysbiosis. *Pharmacological Research* **69**: 75-86.

Willing BP, Dicksved J, Halfvarson J, *et al.* (2010) A pyrosequencing study in twins shows that gastrointestinal microbial profiles vary with inflammatory bowel disease phenotypes. *Gastroenterology* **139**: 1844-1854.

Table 1: Total SCFA concentration (mM) during start-up (day 3 – day 7) and follow-up (day 8 – day 17) period and levels of acetic acid (AA), propionic acid (PA), butyric acid (BA) and branched chain fatty acids (BCFA) during start-up period. Data are means with SE.

		Total SCFA (mM)		AA/PA/BA (%)		BCFA (%)	
		Start-up	Follow-up	Start-up	Follow-up	Start-up	Follow-up
CD 1	C	38.6 (2.8)	39.1 (1.4)	57/15/20 (4/0/2)	45/15/29 (1/0/0)	7 (2)	11 (0)
	T	42.5 (3.3)	42.6 (2.3)	62/15/17 (3/0/2)	48/15/26 (1/0/0)	5 (2)	11 (0)
CD 2	C	58.5 (4.2)	51.8 (2.5)	47/22/19 (2/1/0)	58/20/18 (2/1/0)	9 (1)	4 (1)
	T	56.3 (3.4)	53.1 (2.3)	46/23/20 (1/1/0)	55/21/18 (1/0/0)	10 (0)	7 (1)
CD 3	C	42.3 (2.0)	43.7 (1.1)	65/17/16 (2/0/1)	67/17/13 (1/0/0)	2 (1)	3 (1)
	T	38.9 (2.1)	39.6 (2.1)	62/18/16 (2/0/1)	64/18/15 (1/0/0)	3 (1)	3 (1)
CD 4	C	49.1 (5.2)	48.1 (2.3)	50/20/20 (3/2/1)	50/16/25 (2/0/1)	5 (1)	9 (1)
	T	56.7 (1.8)	51.0 (2.7)	52/17/22 (2/1/1)	53/16/24 (2/0/1)	4 (1)	7 (1)
CD 5	C	45.3 (2.1)	41.5 (1.4)	68/14/13 (0/1/0)	73/17/8 (1/0/0)	4 (0)	2 (0)
	T	43.7 (1.5)	43.7 (2.0)	70/15/12 (1/0/0)	62/16/15 (2/0/1)	2 (0)	6 (1)
HV 1	C	45.3 (3.2)	41.9 (1.8)	73/13/11 (2/0/1)	72/15/8 (1/0/0)	1 (0)	6 (1)
	T	44.8 (2.7)	44.1 (2.1)	71/14/12 (0/0/0)	57/16/19 (2/0/1)	2 (0)	8 (0)
HV 2	C	43.1 (1.9)	39.7 (1.3)	68/13/16 (1/0/1)	58/17/18 (2/0/1)	1 (0)	7 (1)
	T	44.4 (3.1)	42.7 (2.2)	70/14/15 (0/0/0)	58/17/18 (2/0/1)	1 (0)	7 (1)
HV 3	C	46.7 (2.9)	40.6 (1.9)	68/14/13 (1/0/1)	60/17/16 (1/0/1)	4 (0)	8 (1)
	T	42.0 (1.5)	43.1 (1.5)	66/15/17 (1/1/0)	59/14/17 (2/0/1)	1 (0)	8 (0)

FIGURE LEGENDS

Figure 1

Overview of experimental set-up of simulation mucosal and luminal microbiota in M-SHIME®. In total 8 simulations of 17 days were performed starting with fecal microbiota of 8 different individuals. Treatment vessels were supplemented on day 7 with one dose of *B. pullicaecorum* (10^9/mL).

Figure 2

qPCR analysis of mucosal and luminal communities in M-SHIME®.

A) Boxplot of relative abundance of *B. pullicaecorum* (16S rRNA gene copy number *B. pullicaecorum*/ Total 16S rRNA gene) in lumen samples (n = 16) and mucus samples (n = 16) after 7 days in M-SHIME® and before treatment. Black lines within boxplot represent median values and whiskers indicate minimum and maximum value. Means +/- standard error are shown above each boxplot. B) Concentration of *B. pullicaecorum* (log copies/mL or /g) in lumen and mucus samples of control (C) and treatment (T) vessels before treatment (B1), three days after treatment (B2) and ten days after treatment (B3). Each data point represents an individual M-SHIME® sample. Limit of detection (LOD) is indicated by grey horizontal line. Significant differences are indicated by asterisks with ** = $p<0.001$; *** = $p<0.0001$ and ns = non-significant.

Figure 3

Relative concentration of acetate (◆) and butyrate (●) in mol% (Ration mM acetate or butyrate and mM total SCFA) in lumen samples of control (---; open) and treated (—; full)

vessels of M-SHIME®. Dashed vertical line on day 7 indicates single treatment with *B. pullicaecorum* (10^9/mL).

Figure 4

Constrained analysis of principle coordinates based on Bray-Curtis distance from genera composition, constrained by sample type (Lumen vs Mucus) and response (NR vs RS). CAP1 and CAP2 represent the major axes of separations calculated from 'capscale', and NR/RS composes the most primary separation of communities (CAP1) while lumen/mucus samples composes secondary separations (CAP2).

Figure 5

Boxplots of genera that are significantly different between NR and RS control lumen and mucus samples. P-values are from Wilcox test without multiple testing corrections.

Figure 6

Boxplots of genera that are significantly different between treatment and control lumen samples in RS, NR samples were also added to show the differences in response to treatment. P-values are from Wilcox test without multiple testing corrections.

Supplementary Figure 1

Reproducibility of M-SHIME. Fecal sample of two individuals was used to inoculate each two identical M-SHIME colon vessels. SCFA concentrations of lumen fractions for vessel 1 (○, ····) and vessel 2 (●, ---) for IBD 5 (A) and HV 3 (B).

Supplementary Figure 2

qPCR analysis of mucosal and luminal communities in M-SHIME®.

Concentration of Bacteria (log copies/mL or /g) in lumen and mucus samples of control (C) and treatment (T) vessels before treatment, three days after treatment and ten days after treatment. Each data point represents an individual M- SHIME® sample. Limit of detection (LOD) is indicated by grey horizontal line. Significant differences are indicated by asterisks with ** = $p<0.001$; *** = $p<0.0001$ and ns = non-significant.

Supplementary Figure 3

Log difference of *B. pullicaecorum* concentration in treatment and control in lumen and mucus samples after 3 and 10 days of single treatment of different donor microbiota.

Supplementary Figures 4

Constrained analysis of principle coordinates of Bray-Curtis distance based on sample type. The major separation is between faecal and other samples (CAP1) while the separation between lumen and mucus is less prominent (CAP2).

Supplementary Figure 5

Constrained analysis of principle coordinates based on Bray-Curtis distance from genera composition, constrained by sample type (Lumen vs Mucus) and health status (HV vs CD). CAP1 and CAP2 represent the major axes of separations calculated from 'capscale', and lumen/mucus composes the most primary separation of communities (CAP1) while CD/HV samples composes secondary separations (CAP2).

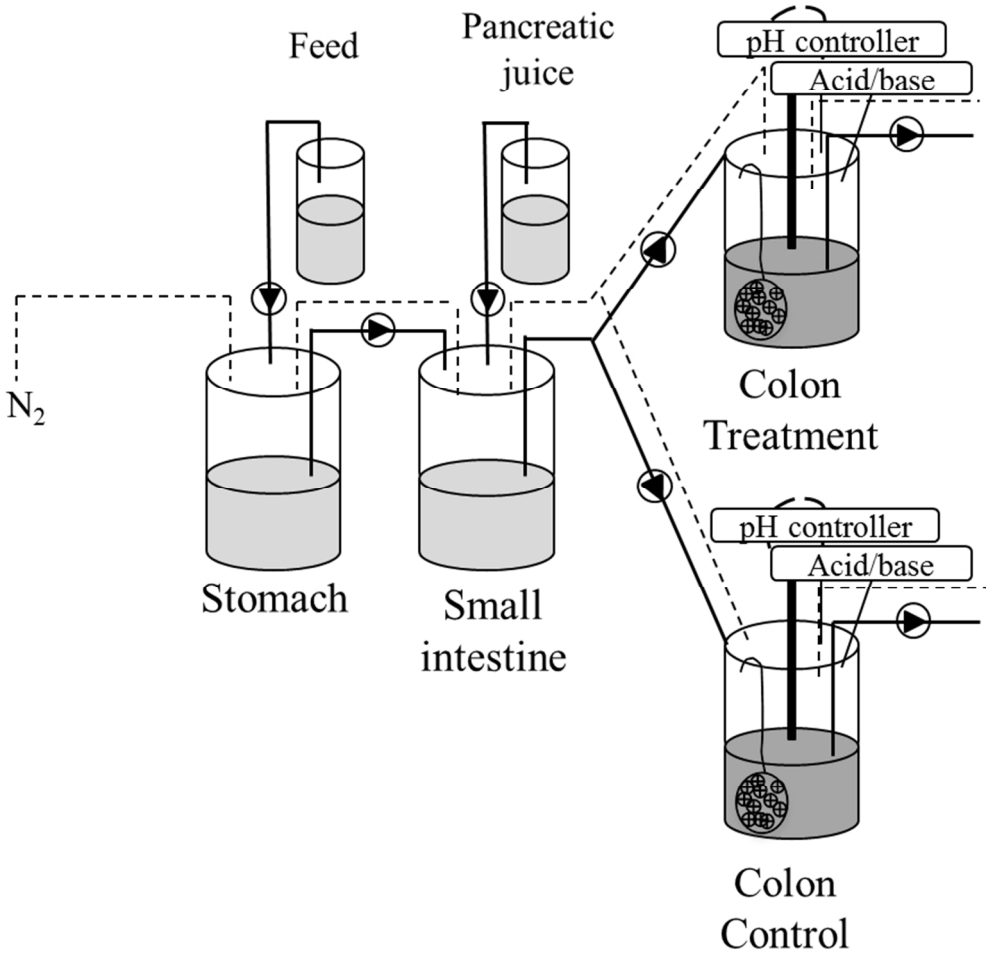


Figure 1
Overview of experimental set-up of simulation mucosal and luminal microbiota in M-SHIME®. In total 8 simulations of 17 days were performed starting with fecal microbiota of 8 different individuals. Treatment vessels were supplemented on day 7 with one dose of B. pullicaecorum (109/mL).
149x149mm (150 x 150 DPI)

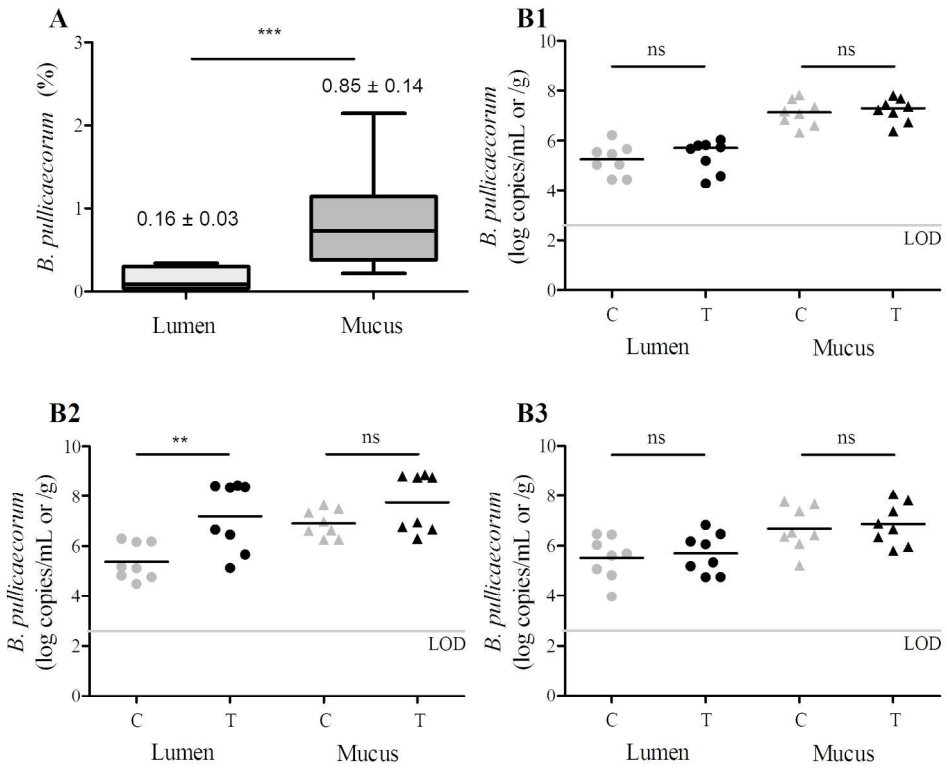


Figure 2
qPCR analysis of mucosal and luminal communities in M-SHIME®.
A) Boxplot of relative abundance of B. pullicaecorum (16S rRNA gene copy number B. pullicaecorum/ Total 16S rRNA gene) in lumen samples (n = 16) and mucus samples (n = 16) after 7 days in M-SHIME® and before treatment. Black lines within boxplot represent median values and whiskers indicate minimum and maximum value. Means +/- standard error are shown above each boxplot. B) Concentration of B. pullicaecorum (log copies/mL or /g) in lumen and mucus samples of control (C) and treatment (T) vessels before treatment (B1), three days after treatment (B2) and ten days after treatment (B3). Each data point represents an individual M- SHIME® sample. Limit of detection (LOD) is indicated by grey horizontal line. Significant differences are indicated by asterisks with ** = $p<0.001$; *** = $p<0.0001$ and ns = non-significant.
202x164mm (300 x 300 DPI)

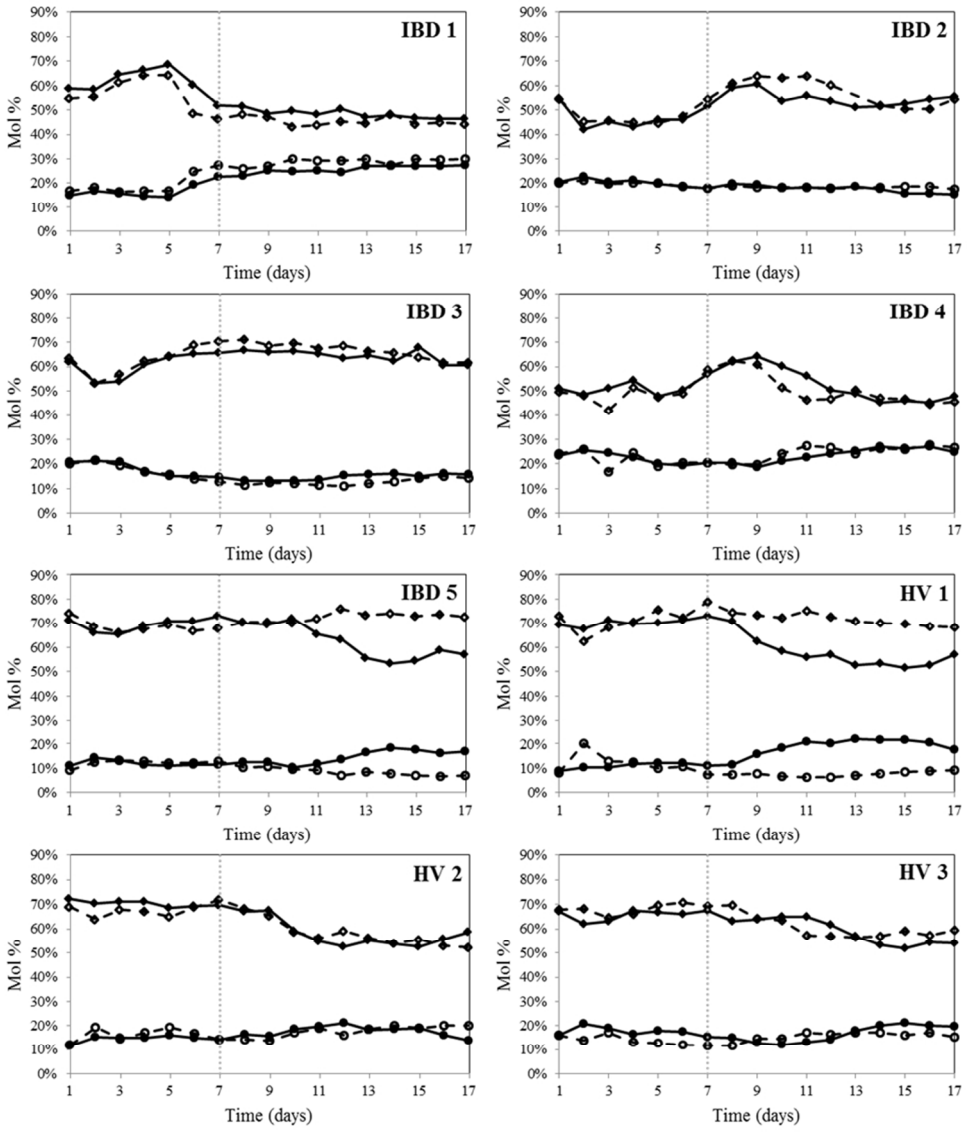
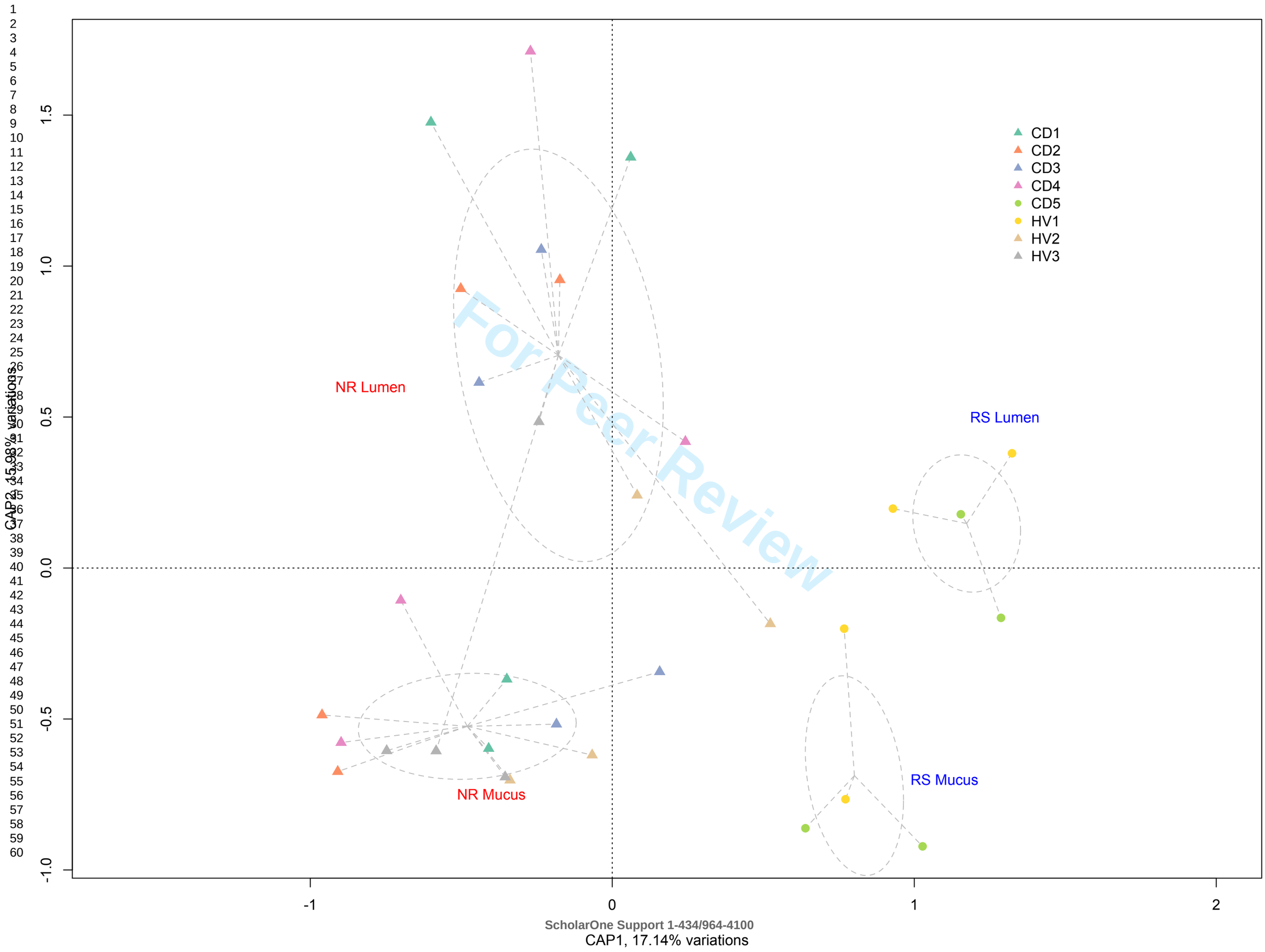
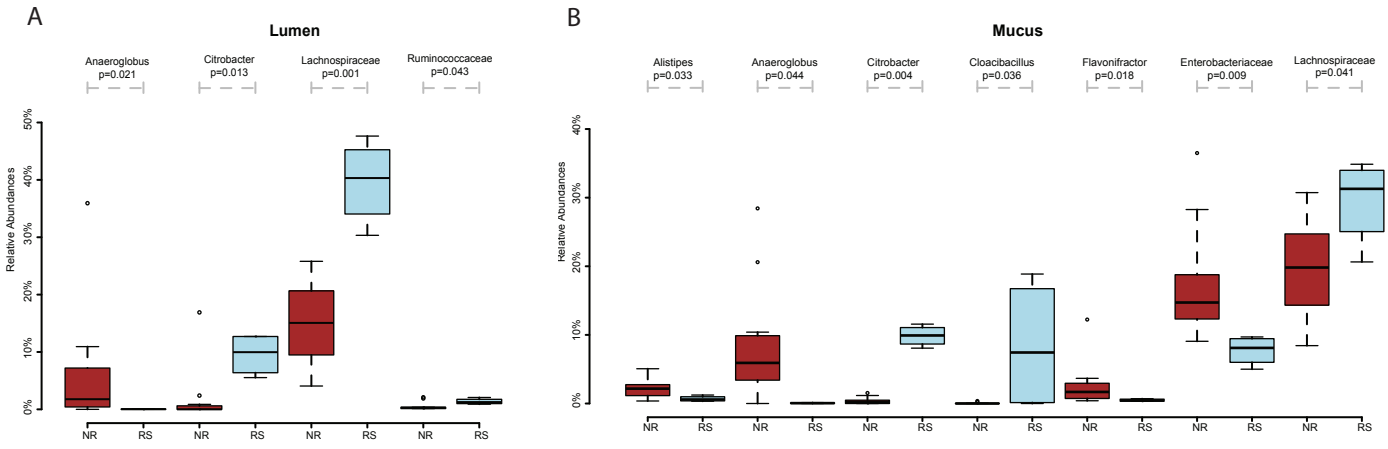
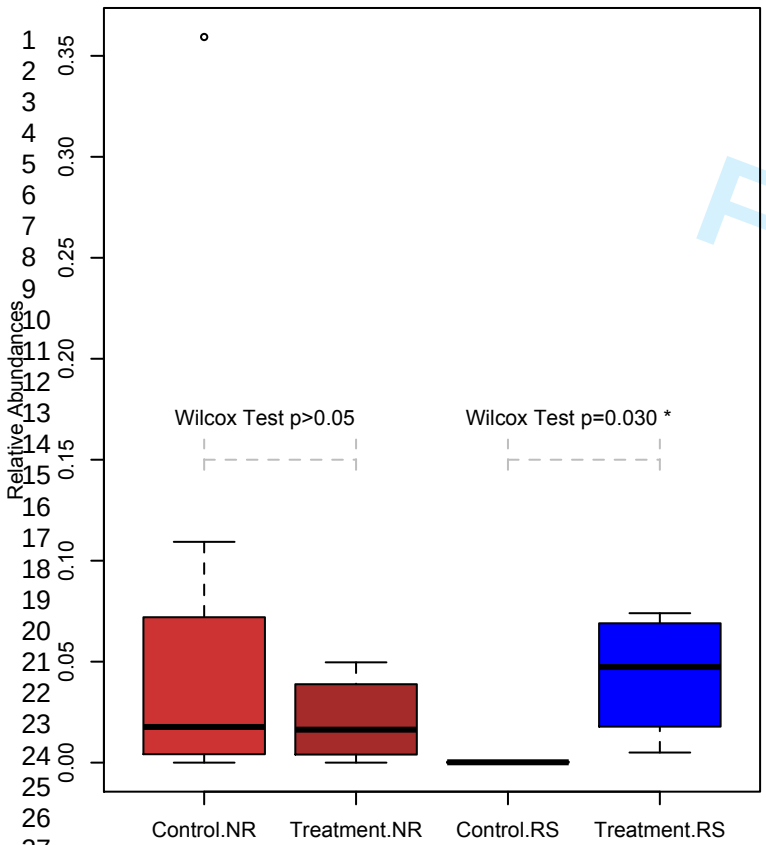
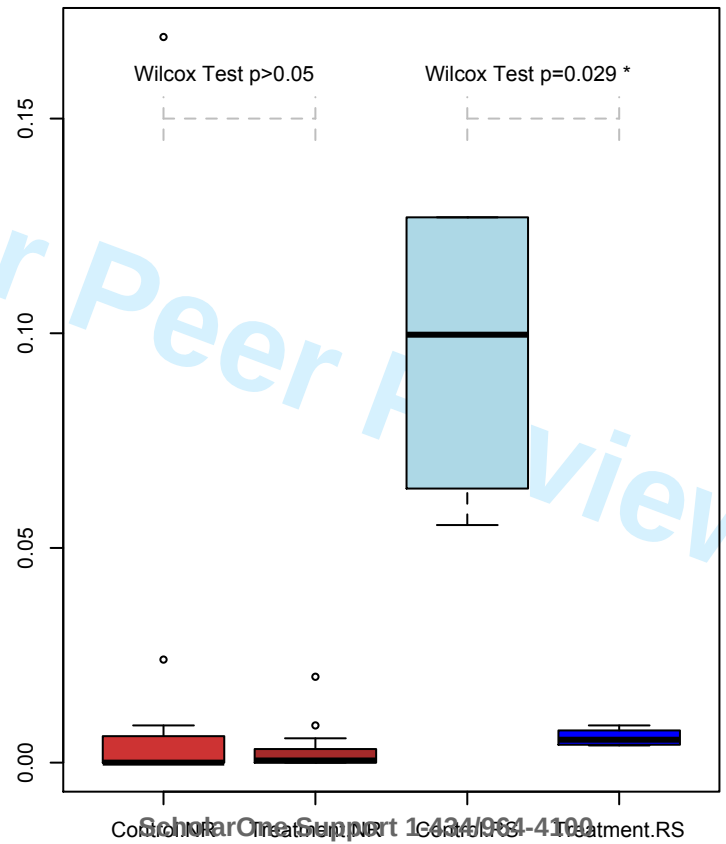


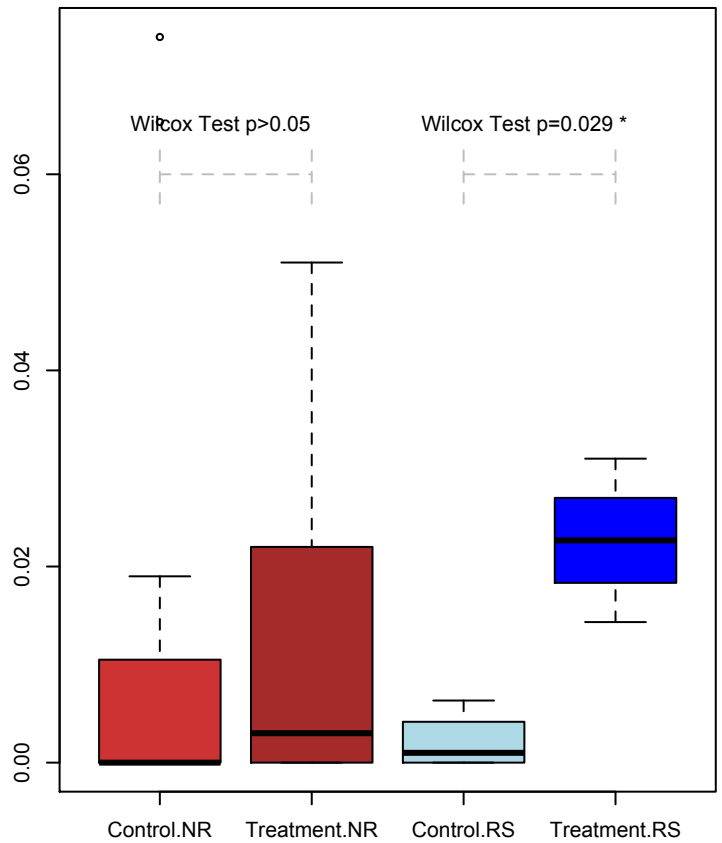
Figure 3
Relative concentration of acetate (→) and butyrate (●) in mol% (Ration mM acetate or butyrate and mM total SCFA) in lumen samples of control (---; open) and treated (—; full) vessels of M-SHIME®. Dashed vertical line on day 7 indicates single treatment with B. pullicaecorum (109/mL).
168x195mm (150 x 150 DPI)











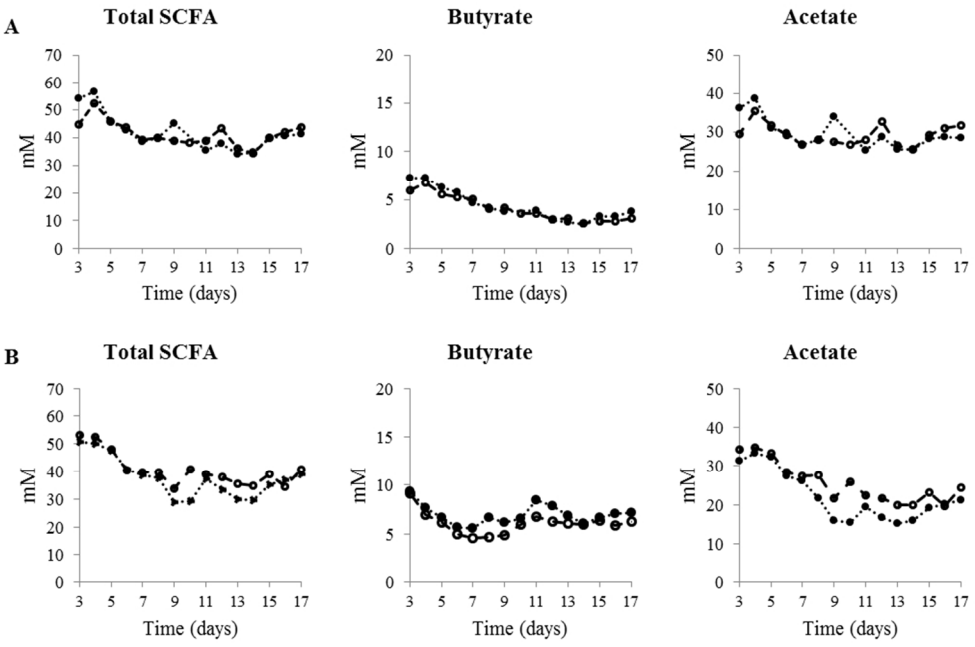
SUPPLEMENTARY INFO

A minimum of 3,000 reads was obtained for each sample included in the study, and the composition resembles that of typical human fecal/gut microbiome. At phylum level, Firmicutes (44.7+-16.4%), Proteobacteria (23.3+-11.8%) and Bacteroidetes (22.4+-17.7%) are the major phyla; while at genus level, the unclassified_Lachnospiraceae (18.4+-9.8%), unclassified_Enterobacteriaceae (10.9+-10.2%), and *Bacteroides* (15.2+-13.1%) are respectively the major genera of the dominant phyla.

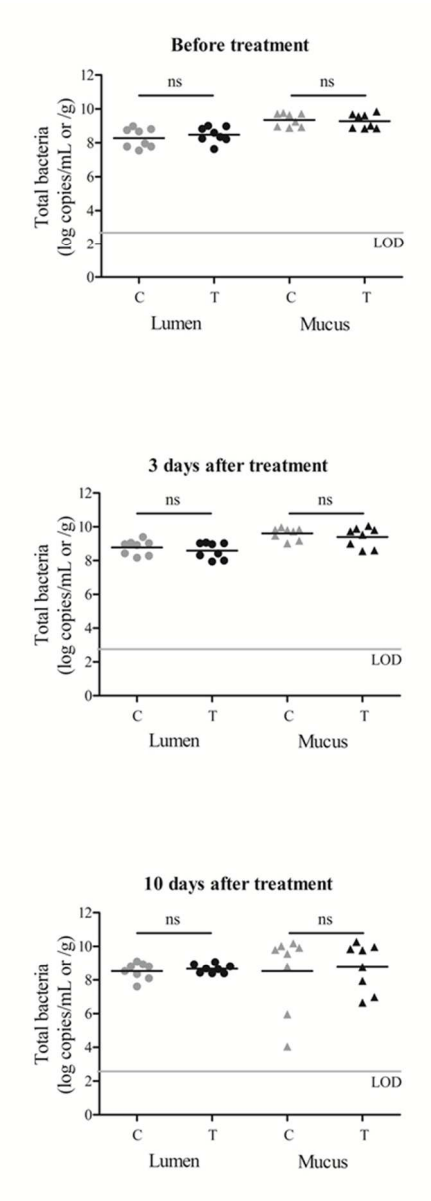
We examined the relative contribution of different variables that could contribute to the variations of the microbiome (Bray-Curtis dissimilarities based on genera composition) using "adonis". Donor individuals have the largest effect on the microbiome and explain 32.7% of community variations based on genus composition (p=0.001). Type of samples (fecal, mucus and lumen) has second largest effect (15.2% variation, p=0.001), and thus different samples were analyzed separately, with a focus on lumen and mucus samples. (Supplementary figure 4). The disease status (healthy versus CD) has smaller yet significant effect in mucus (12.5% variation, p=0.003) and lumen (11.8% variation, p=0.002), but not significant in fecal samples possibly due to the small sample size (n=8) (Supplementary figure 5). Control versus treatment and time since treatment do not have significant effect on the microbial composition alone or combined (all p>0.05), and no significance is found when they are limited to lumen or mucus samples (all p>0.05).

Supplementary Table 1: Relative abundance of genus *Butyricicoccus* (16S rRNA gene amplicon sequencing)

		***Butyricicoccus* spp.**			
		3 days after treatment		7 days after treatment	
		Mucus	Lumen	Mucus	Lumen
CD 1	C	5	3	3	12
	T	30	27	3	3
CD 2	C	0	1	1	2
	T	2	2	1	0
CD 3	C	6	6	4	9
	T	3	9	3	9
CD 4	C	2	5	11	0
	T	21	28	6	1
CD 5	C	0	2	3	1
	T	106	360	14	1
HV 1	C	4	6	2	1
	T	113	321	15	2
HV 2	C	7	15	7	5
	T	207	458	17	4
HV 3	C	6	3	5	1
	T	124	385	12	1

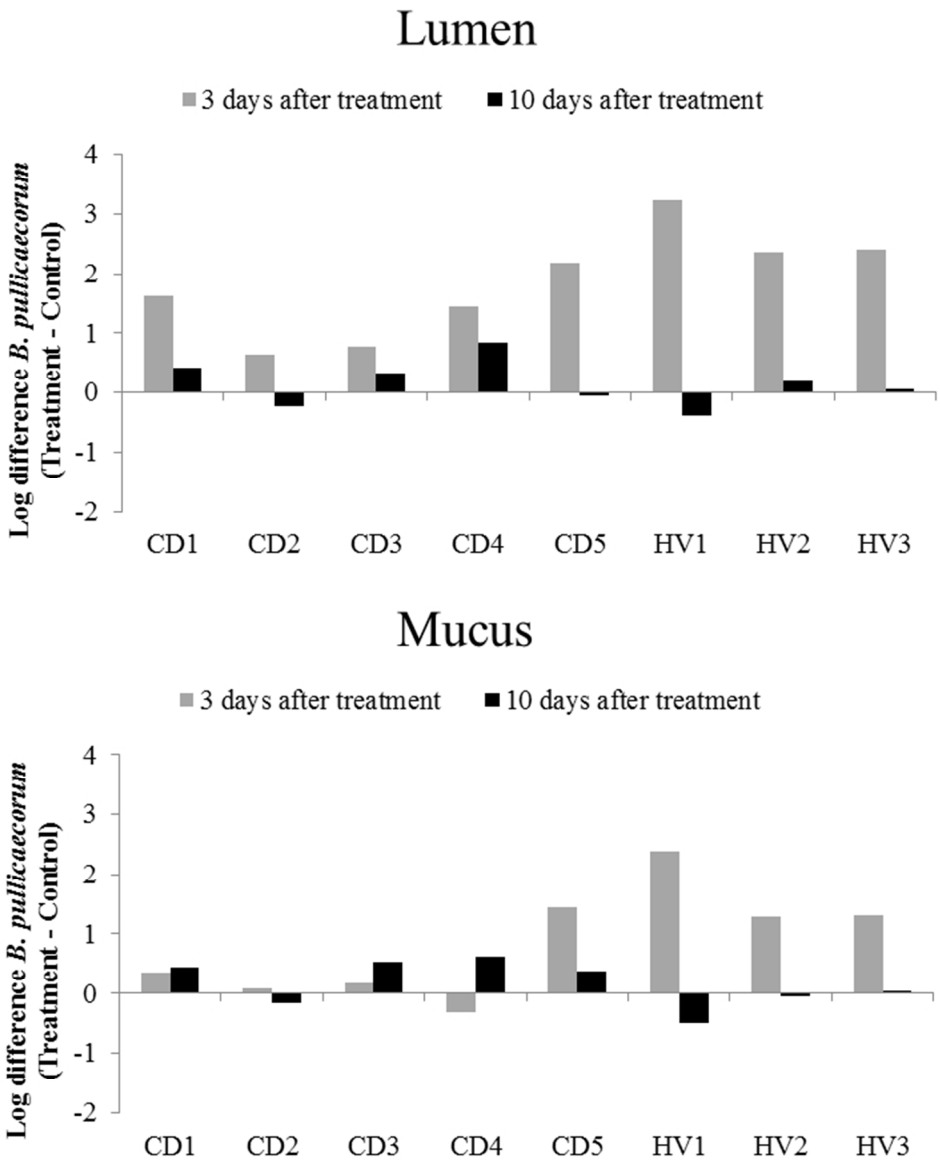


Supplementary Figure 1
Reproducibility of M-SHIME. Fecal sample of two individuals was used to inoculate each two identical M-SHIME colon vessels. SCFA concentrations of lumen fractions for vessel 1 (○, ...) and vessel 2 (●, ---) for IBD 5 (A) and HV 3 (B).
176x119mm (150 x 150 DPI)



Supplementary Figure 2
qPCR analysis of mucosal and luminal communities in M-SHIME®.
Concentration of Bacteria (log copies/mL or /g) in lumen and mucus samples of control (C) and treatment (T) vessels before treatment, three days after treatment and ten days after treatment. Each data point represents an individual M- SHIME® sample. Limit of detection (LOD) is indicated by grey horizontal line. Significant differences are indicated by asterisks with ** = $p<0.001$; *** = $p<0.0001$ and ns = non-significant.

69x190mm (150 x 150 DPI)



Supplementary Figure 3
Log difference of B. pullicaecorum concentration in treatment and control in lumen and mucus samples after 3 and 10 days of single treatment of different donor microbiota.
127x152mm (150 x 150 DPI)